iPSC Handbook

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Introduction

The expression of the four human transcription factors (TFs) (Yamanaka Oct4, Klf4, Sox2, and c-Myc) or (Thomson Oct4, Sox2, Nanog, and Lin28) have been shown to reprogram different types of terminally differentiated cells to an embryonic stem (ES) cell-like state known as the induced pluripotent stem (iPS) cells (Yu, et al., 2007; Takahashi, et al., 2007). **abm** has developed the most comprehensive product line for iPSC generation and related stem cell research areas. This includes iPSC purified recombinant proteins, minicircle DNA, iPSC lentiviral and adenoviral particles, and EBV-based non-integrated plasmids. With those tools available, you can generate iPSC from any cell type with choices of both transient (proteins, adenovirus, and plasmids) and stable (lentivirus) iPSC factors. In addition, choices of iPSC factors driven by different promoters in lentivirus (CMV, UBC, PGK, and EF1a) are also available. We have a greater selection of iPSC vectors than any other provider in stem cell research.

Stringent Quality Testing

abm's application testing ensures the highest quality of reprogramming recombinant proteins, lentiviruses, adenoviruses, and plasmids. Every lot of products have been confirmed to successfully produce iPSCs or to express specific iPSC factors in cell types. With proven quality, you can minimize the need to repeat experiments and advance your project efficiently.

Choices for Different Formats, Concentrations, and Species

abm's broad iPSC product line enables researchers to select the right product applicable to their research – no need to compromise. Products are provided in th e formats of recombinant proteins, viral particles, and plasmids for both human and mouse iPSC factors. Custom development of any specialized iPSC products is also available. All known six reprogramming factors are available as individual viruses and in functional sets.

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Minicircle iPSC DNA

The minicircle DNA (mc-DNA) is an independent mammalian expression cassette void of any bacterial DNA element required for plasmid production. Because of this, the expression cassette has been shown to have an extended life span with higher and longer expression levels of over 3 weeks in different cells studied (Chen, et al., 2003). In the end, the non-integrating mc-DNA gets degraded and loses the expression of target gene. Thus it is ideal for the application of iPSCs due to its higher efficiency than iPSC proteins and the non-integrating nature as compared to lentiviral vectors.

As mc-DNA is a good choice for iPSC induction, competitors now offer similar products with CMV promoter which is of viral origin and is the target for methylation. The methlylation of CMV promoter is often the reason leading to gene silencing. Thus, mc-DNAs with CMV promoter will significantly compromise efficacy of iPSC induction. The ideal choice for mc-DNA iPSC factors is Xeno-free DNA with promoters of human or mouse origin. Promoters like EF1a should therefore be the choice, which has proven to be the choice of promoter for iPSC applications in lentiviral vectors.

Figure 1. iPSC minicircle DNA map

Table 1. Product List of Minicircle iPSC DNA

Product Name	Cat. No.	Description	Size
iPSC-Minicircle (Human)	G388	Minicircle DNA-Yamanaka	100 µg
	G389	Minicircle DNA-Thomson	
iPSC-Minicircle (Mouse)	G390	Minicircle DNA-mYamanaka	100 µg
	G391	Minicircle DNA-mThomson	

Recombinant Human iPSC Proteins

One of the major goals of iPSCs research is for disease modeling and novel therapeutic development. Insertion of any genetic materials into the newly generated iPSCs other than iPSC transcription factors is a grave concern for accurate disease modeling and therapeutic development. Protein-based human iPSCs technology is the ultimate choice for iPSCs that does not require the destruction of ex utero embryos and completely eliminates possible genome mutations and insertions associated with DNA transfection or viral transduction.

Recombinant iPSC Protein Lysates

Reports from two independent laboratories clearly demonstrated the feasibility of iPSC generation with crude recombinant protein lysates (Kim, et al., 2009; Zhou, et al., 2009). The following human iPSC reprogramming factors are produced by transfection in 293 cells with poly-Arginine protein penetrating domain (PPD) at the C-terminal end of each protein that allows protein transduction of any cell type efficiently.

Purified Recombinant iPSC Proteins

Though crude reprogramming protein lysates was shown to be successful in iPSC generation, the efficiency is extremely low. Purified iPSC from a mammalian expression system would be the ideal choice. However, this has proven to be extremely challenging. After vigorous trials and errors, scientists at **abm** is able to purify all human iPSC proteins from human cells with proven functionality. All iPSC proteins are expressed from 293 cells fused with His tag at N-terminal for purification and poly-Arginine (PPD) at C-terminal for cell transduction.

Table 2. Product List of Purified Recombinant iPSC Proteins

Product Name	Cat. No.	Description	Size
iPSC-Protein Sets	000025p	Thomson Set (purified from 293 cells)	5X50 µg
	000027p	Yamanaka Set (purified from 293 cells)	
iPSC-Klf4 Protein	000001p	Purified from 293 cells	50 µg
iPSC-Lin28 Protein	000003p	Purified from 293 cells	50 µg
iPSC-Myc Protein	000005p	Purified from 293 cells	50 µg
iPSC-Nanog Protein	000007p	Purified from 293 cells	50 µg
iPSC-Oct4 Protein	000009p	Purified from 293 cells	50 µg
iPSC-Sox2 Protein	000011p	Purified from 293 cells	50 µg

Protein Transfection Reagents (Cat. G288)

One of key challenges in protein-based iPSC generation is the low efficiency of protein transduction of target cells. Though a number of protein translocation domains including TAT, poly Arg, and others have been shown to be useful in protein transduction, but the efficiency is still relatively low. To this end, we have developed optimized protein transfection reagents that can enhance protein transduction efficiency by up to 70%.

iPSC Lentiviral Particles

Lentiviral particles are the most efficient vectors for stable transduction of any cell type. Recombinant iPSC lentiviruses would be the ideal tool for efficient iPSC generation. **abm** now provides the most comprehensive iPSC reprogramming lentiviruses including all 6 iPSC genes (both mouse and human) with choices of EF1a, PGK, UBC, and CMV promoters. In addition, a GFP control virus is developed for each of the promoters listed above, which allows quick evaluation of an individual promoter's strength in the target cells of interest. All lentiviruses are purified at high titer of $1X10^7$ cfu/ml and VSV-G pseudotyped for efficient transduction of a wide range cell types.

Table 3. Strength of Commonly Used Mammalian Promoters

Promoter	Expression level	Applications
CMV	High	-Very strong promoter in mammalian cells -Prone to DNA methylation and not applicable in stem cell cells
EF1a	High	-Strong promoter in mammalian cells -Applicable to stem cells
PGK	Medium	-Medium promoter strength -Relative strong in stem cells, but transduction efficiency is 85% of EF1a promoter under the same condition.
UbC	Low	-Relatively weaker promoter -Longer gene expression up to 8 weeks -Applicable to stem cells

Table 4. iPSC Lentiviral particles

Product Name	Cat. No.	Description	Size
Lenti-III-EF1a-Klf4	G333	Viral particles ($1X10^7$ cfu/ml)	200 µl
Lenti-III-EF1a-Lin28	G334	Viral particles ($1X10^7$ cfu/ml)	200 µl
Lenti-III-EF1a-Nanog	G335	Viral particles ($1X10^7$ cfu/ml)	200 µl
Lenti-III-EF1a-Oct4	G336	Viral particles ($1X10^7$ cfu/ml)	200 µl
Lenti-III-EF1a-Myc	G337	Viral particles ($1X10^7$ cfu/ml)	200 µl
Lenti-III-EF1a-Sox2	G338	Viral particles ($1X10^7$ cfu/ml)	200 µl
Lenti-III-EF1a-GFP	G385	Viral particles ($1X10^7$ cfu/ml)	200 µl

Table 5. iPSC Lentivirus Sets

Product Name	Cat. No.	Description	Size
iPSC Lentivirus set (Human)	G356	Lenti-III-Thomson Set (OSLN and GFP)	5 X 200 µl Viral particles ($1X10^7$ cfu/ml)
	G361	Lenti-III-Yamanaka Set (OSKM and GFP)	
iPSC Lentivirus set (Mouse)	G371	Lenti-III-mThomson Set (OSLN and GFP)	5 X 200 µl Viral particles ($1X10^7$ cfu/ml)
	G372	Lenti-III-mYamanaka Set (OSKM and GFP)	

iPSC Polycistronic Lentiviral Particles

The polycistronic lentiviruses express each of the four transcription factors (hOSKM: Oct4, Sox2, cMyc or Klf4) or (hOSLN: Oct4, Sox2, Lin28, Nanog) of human and mouse from a single expression cassette under the control of EF1a promoter. Each of the factors is separated by a unique 2A peptide (P2A, T2A, and E2A) sequence, which allows for equivalent expression of all four transcription factors from a single vector. (Carey, et al., 2009; Shao, et al., 2009). The expression of 4 iPSC factors from a single virus minimizes the number of proviral integrations required for successful reprogramming. This also reduces risks of insertional mutagenesis effects in iPSCs as compared to those generated with four independent viruses. The viruses are provided as concentrated and VSV-G pseudotyped stock capable of transducing both dividing and non-dividing cells.

Table 6. iPSC Polycistronic Lentiviral Particles

Product Name	Cat. No.	Description	Size
Lenti-III-EF1a-OSNL	G351	Polycistronic viral particles $1X10^7$ cfu/ml	200 µl
Lenti-III-EF1a-OSKM	G352	Polycistronic viral particles $1X10^7$ cfu/ml	200 µl
Lenti-III-EF1a-mOSNL	G369	Polycistronic viral particles $1X10^7$ cfu/ml	200 µl
Lenti-III-EF1a- mOSKM	G370	Polycistronic viral particles $1X10^7$ cfu/ml	200 µl

iPSC Adenoviruses

Adenoviral particle is the most efficient gene delivery vehicle for mammalian cell transduction. Almost 100% transduction efficiency can be achieved in most human and mouse cells *in vitro*. In addition, adenoviral particles do not integrate into the target cell genome, giving rise to only transient transgene expression. This makes it a very attractive choice for iPSCs generation, eliminating possible genetic mutations associated with vector integration.

Table 9. iPSC Adenoviral Particles

Product Name	Cat. No.	Description	Size
Ad-SOX2	000691A	Viral particles ($1X10^{11}$ pfu/ml)	250 µl
Ad-OCT4	000692A	Viral particles ($1X10^{11}$ pfu/ml)	250 µl
Ad-KLF4	000693A	Viral particles ($1X10^{11}$ pfu/ml)	250 µl
Ad-c-MYC	000695A	Viral particles ($1X10^{11}$ pfu/ml)	250 µl
Ad-LIN28	000696A	Viral particles ($1X10^{11}$ pfu/ml)	250 µl
Ad-NANOG	000697A	Viral particles ($1X10^{11}$ pfu/ml)	250 µl

Table 10. Polycistronic Adenoviral Particles

Product Name	Cat. No.	Description	Size
Ad-hOSLN (human)	000777A	Viral particles ($1X10^{11}$pfu/ml)	5X250µl
Ad-hOSKM (human)	000779A		
Ad-mOSLN (mouse)	000787A	Viral particles $1X10^{11}$pfu/ml	5X250µl
Ad-mOSKM (mouse)	000789A		

iPSC Protocols:

The following protocol offers a general guideline for the induction of iPSCs from fibroblasts. Appropriate modifications need to be developed for cells that require unique culture conditions.

General Reagents required:

HDFf medium: DMEM (10%FBS, 1% Non-essential amino acid)
iPSC medium: DMEM (20% knock-out serum replacer, 200mM L-glutamine, 1% Non-essential amino acid, 8 ng/ml b-FGF)

iPSC Adenovirus Protocol

Before any experiment, make sure to test your cell's transduction efficiency with a GFP reporter virus so that the right titer of virus can be used for your cells of interests. The following procedure is based on human fibroblasts and will also be applicable to other cell types.

1. Seed HDF cells in 10cm dishes (20-30% confluent) one day before viral infection, including one dish used for GFP control.
2. The following day, dilute 50 µl of each adenovirus (individual one or polycistronic ones) in 4ml complete culture medium.
3. Aspirate medium from 10cm dishes and add diluted virus (4 ml) to cells. Return dishes to a CO_2 incubator for one hour.
4. Aspirate virus-containing medium, and replenish with 10 ml iPSC medium.
5. Change medium every 48 hours.
6. Repeat adenoviral gene transduction every other 5 days as transgene expression by adenovirus will only last 5~7 days, depending on the rate of cell division. Longer expression can be expected for cells with slower cell dividing rates.
7. Wait for 2-4 weeks for iPSC colonies to form.
8. Once iPSC colonies form, prepare Mytomycin C treated MEF (mouse embryonic fibroblasts) feeder cells in a 24-well plate (80% confluent) at a concentration of 10µg/ml for 3 hours in an incubator at 37°C followed by 2X PBS wash.
9. Pick colonies manually into 96-well plate and trypsinize in 96-well plate.
10. Transfer the trypsinized cells from each of the 96 wells into 24-well MEF coated plate.
11. Wait for another 1-2 weeks for iPSC colonies to develop (change medium every 48 hours)
12. When MEF cells become too old (about 2 weeks) or a lot of iPSC colonies have developed in the 24-well plate, prepare MEF feeder layer as described above in 6-well plate to expand.
13. Trypsinize the cells (both MEF and iPSC cells) and spin down at 1000Xg.
14. Seed iPSCs into 6-well MEF coated plate for expansion.
15. Using the same procedures above, iPSCs can be further expanded to bigger culture vessels to meet your lab needs for iPSC analysis or down-stream applications.

iPSC Lentivirus Protocol

Recombinant iPSC lentiviral particles (either set or polycistronic particles) from **abm** are purified by high titer preparation and are ready to use. The following protocol is based on human fibroblasts and will be applicable to most other cell types.

1. Seed HDF 1X10^5 cells in a 6 well plate (~70% confluent) one day before viral infection, including one dish used for GFP control.
2. The following day, dilute 10~50µl of each lentivirus (individual one or polycistronic ones) to 0.5ml complete culture medium at 3:00pm in the afternoon.
3. Aspirate medium from 6-well plate and add diluted virus (0.5ml) to the 6-well plate and add polybrene at a concentration of 2 µg/ml. Return the plate to a CO_2 incubator for overnight incubation.

Note: *Polybrene is a polycation that neutralizes charge interactions to increase binding between the pseudoviral capsid and the cellular membrane. The optimal concentration of Polybrene depends on cell type and may need to be empirically determined (usually in the range of 1–8µg/ml). Excessive exposure to Polybrene (>12 hr) can be toxic to some cells.*

4. The following day, aspirate virus-containing medium and replenish with 2 ml iPSC medium.
5. Change medium every 48 hours.
6. Wait for 2-4 weeks for iPSC colonies to form.
7. Once iPSC colonies form, prepare Mytomycin C treated MEF (mouse embryonic fibroblasts) feeder cells in 24-well plate (80% confluent) at a concentration of 10 µg/ml for 3 hours in an incubator at 37°C followed by 2X PBS wash.
8. Pick colonies manually into 96-well plate and trypsinize in 96-well plate.
9. Transfer the trypsinized cells from each of the 96 wells into 24-well MEF coated plate.
10. Wait for another 1-2 weeks for iPSC colonies to develop (change medium every 48 hours)
11. When MEF cells become too old (about 2 weeks) or a lot of iPSC colonies have developed in the 24-well plate, prepare MEF feeder layer as described above in 6-well plate to expand.
12. Trypsinize the cells (both MEF and iPSC cells) and spin down at 1000Xg.
13. Seed iPSCs into 6-well MEF coated plate for expansion.
14. Using the same procedures above, iPSCs can be further expanded to bigger culture vessels to meet your lab needs for iPSC analysis or down-stream applications.

iPSC mc-DNA Protocol

iPSC induction with mc-DNA requires transfection reagents with high transduction efficiency and low toxicity. Unfortunately, transduction efficiency with transfection reagents in most primary cells are very low and minimal 20% efficiency is required for successful iPSC generation. The transfection efficiency can be determined with a GFP reporter. In most cases, our DNAfectin 2100 (Cat. No. G2100) works well in most primary cells. In general, reprogramming requires approximately 4µg per transfection per well in 6 well plate three times.

1. Seed HDF $1X10^5$ cells in a 6 well plate (~70% confluent) one day before transfection, including one dish used for GFP control.
2. In a 1 ml Eppendoff tube A, dilute 4 µg of mc-DNA in 100 µl of medium without serum. In another tube B, dilute 10µl of DNAfectin 2100 in 100µl of medium without serum. Mix gently and incubate the two tubes for 5 min at room temperature.
3. Combine the medium in tube A and B, mix gently and incubate for another 20 minutes at room temperature.
4. Add 800 µl serum-free medium to the complexes followed by gentle mixing.
5. Remove the medium from the cells, wash with PBS once and add the ~1 ml complexes from the last step to the 6-well without dislodging cells. Incubate the cells for 5 hours at 37°C.
6. After 5 hours, add 100µl serum replacer to the culture dish. Incubate overnight.
7. The following day, replace the medium with 2 ml fresh iPSC medium.
8. Repeat mc-DNA transfection for at least 3 times to sustain activity of reprogramming mc-DNA for 7-14 days in the cells for the reprogramming process.
9. Colonies with morphologies similar to hESC colonies are clearly visible by day 18 after the initial transfection.
10. Once iPSC colonies form, prepare Mytomycin C treated MEF (mouse embryonic fibroblasts) feeder cells in 24-well plate (80% confluent) at a concentration of 10 µg/ml for 3 hours in an incubator at 37°C followed by 2X PBS wash.
11. Pick colonies manually into 96-well plate and trypsinize in 96-well plate.
12. Transfer the trypsinized cells from each of the 96 wells into 24-well MEF coated plate.
13. Wait for another 1-2 weeks for iPSC colonies to develop (change medium every 48 hours)
14. When MEF cells become too old (about 2 weeks) or a lot of iPSC colonies have developed in the 24-well plate, prepare MEF feeder layer as described above in 6-well plate to expand.
15. Trypsinize the cells (both MEF and iPSC cells) and spin down at 1000Xg.
16. Seed iPSCs into 6-well MEF coated plate for expansion.
17. Using the same procedures above, iPSCs can be further expanded to bigger culture vessels to meet your lab needs for iPSC analysis or down-stream applications.

iPSC Protein Protocol

The following protocol is based on human fibroblasts and will be applicable to most other cell types.

1. Seed HDF cells at $5x10^4$ cells per well in a 6-well plate. On the next day, media was changed to the protein transduction media, which was prepared by mixing the recombinant reprogramming proteins at the final concentration of 32µg/ml (8 µg/ml per factor) with iPSC medium (protein transfection reagent G288 is highly recommended to use for a higher transduction efficiency).
2. After overnight culture in the protein transduction media, media was changed to normal iPSC medium, and cells were cultured for additional 36 hours before repeating the same protein transduction cycle.
3. Repeat protein transduction for at least 4 times to sustain activity of reprogramming proteins for 7-10 days in the cells for the reprogramming process.
4. Change medium every 48 hours.
5. Once iPSC colonies form, prepare Mytomycin C treated MEF (mouse embryonic fibroblasts) feeder cells in 24-well plate (80% confluent) at a concentration of 10 µg/ml for 3 hours in an incubator at 37°C followed by 2X PBS wash.
6. Pick colonies manually into 96-well plate and trypsinize in 96-well plate.
7. Transfer the trypsinized cells from each of the 96 wells into 24-well MEF coated plate.
8. Wait for another 1-2 weeks for iPSC colonies to develop (change medium every 48 hours)
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10. Trypsinize the cells (both MEF and iPSC cells) and spin down at 1000Xg.
11. Seed iPSCs into 6-well MEF coated plate for expansion.
12. Using the same procedures above, iPSCs can be further expanded to bigger culture vessels to meet your lab needs for iPSC analysis or down-stream applications.

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